Project No. ______
Book No. 10974

E ______

Page No.

To test in vitro / invivo effect of noradrenergic agonists on splenic lymphocyte / cytokine following LPS activation; follow w/ in vivo / splenic Stim or Sympathectomy

Experimental Design

* To determine if elect stim of the Splenic nerve modulates immune responses

IN VITRO

rat → Spleen → Splenic mø / Splenic lymphocytes / mixed pop

LPS → 2, 6, 8 hrs

NE agonist +/- antagonist (see Maes et al, 2000 for relative doses)

→ Measure in Sups: TNFα, IL-1, IL-6, IL-10, GM-CSF?

Collect sups for initial levels?

IN VIVO

* Electrical Stimulation OR Sympathectomy by Cut Splenic nerve OR 6OHDA IP or Saline

Rat → (LPS IP / IV or LPS IP / IV) → 2, 6, 8 hrs → - - - → Measure viability or

→ take Serum levels of TNFα, IL-1, IL-6, IL-10

→ take spleen, tissue levels of lung? liver? mRNA? by PCR or rt PCR? or Intracellular staining → FACS

- Could also look @ Myeloperoxidase activity + apoptosis
- Could use animal models of various diseases EAE?

* It would be important to do various stim parameters to determine the effects on immunomodulation

it is feasible that different parameters would produce different outcomes - possibly opposite??

* Could be that interaction w/ one type of receptor do . thing + the other type (α or β) does the opposite ∴ may administer antagonist to the one that produce undesired effect

(left margin:) pers (2002) / ids @ receptors / cytokine pro / ints w/ / h Arthritis but / ealthy Patients

To Page No. ___

Witnessed & Understood by me. [signature] Date Redacted

Invented by [signature] Date Redacted

Recorded by

Electrical Stim to the spleen

Rational:

- No cholinergic nerve terminals & virtually no afferent supply in the spleen of rats. (Felton, Cohen & Bellinger each publish in this area)

- Splenic nerve is considered to consist mainly of sympathetic fibers (noradrenergic)
- Sympathectomy sig ↓ the hemorrhage-induced rise in lung TNF in response to hemorrhage (Molina, 2001)
- Stim of β adrenoreceptors in vitro inhibits blastogenic response of lymphocytes to T & B cell mitogens, IL-2 induced proliferation of lymphocytes, lytic activity of cytotoxic T cells & T cell dependent Ab production

- However, other labs report enhancement in lymphocyte proliferation, lytic activity & antibody responses by β-adrenergic stimulation (Hatfield, M. Sanders)

Margin notes: suggest a major role for adrenergic innervation

discrepancies are likely due to dose response
at low levels
↑ adrenoreceptors
lymphocyte prolif
high levels
↓ lymphocyte prolif

I Propose:

- electrical stimulate w/ implantable device @ the splenic nerve to activate the β receptors on lymphocytes to enhance immune response

To silence sympathetic, may have to do hi frequency? DC?
But, it may not be necessary to silence, on/off immunosuppression would benefit several diseases.

To Page No.........

Read & Understood by me, Mark [signature]
Date Redacted
Invented by Lisa [signature]
Recorded by
Date Redacted